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THE MORPHOLOGY OF *PHYLLOCLADUS ALPINUS*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 118

N. JOHANNA KILDAHL

(WITH PLATES XX–XXII)

The only account of the morphology of Phyllocladus is that of Miss AGNES ROBERTSON (**18**) in 1906, and this deals exclusively with the anatomical structures and with the affinities of the genus.

The material for the present study was collected by Dr. L. COCKAYNE of New Zealand. It consisted of two collections: one of ovulate and staminate strobili, collected November 1, 1906; the other of ovulate strobili, collected December 11, 1906, from cultivated trees. Unfortunately, the two collections did not afford a very close series, and it was impossible to get at some of the most important points, as the development of the microsporangia, microspores, pollen tubes, female gametophyte, archegonial neck cells, ventral cell or nucleus, and embryo. Dr. COCKAYNE is making collections of the lacking stages at the present time, and it is hoped that a complete description can be presented later. Some material was also obtained from Cambridge, Mass., through the kindness of Dr. B. L. ROBINSON, consisting of three ovulate cones, taken from the Gray Herbarium and collected on the Wilkes expedition in 1838–1842. At what time of the year these cones were collected is not known, but they showed somewhat later stages than those of the last collection made by Dr. COCKAYNE on December 11. It is needless to say that this herbarium material was very much shrunken, and it was impossible to obtain from it any definite structure.

Dr. COCKAYNE'S material was killed and fixed in the field, in 70 per cent. alcohol and formalin; cut 5 μ and 7 μ thick; and stained part in safranin and gentian violet, and part in iron alum.

Staminate strobilus

The staminate strobili are formed laterally, in clusters of 2–8 at the tips of the lateral branches. Miss ROBERTSON (**18**) reports one staminate strobilus bearing an ovule at its base, which may indicate that the ancestral condition was bisporangiate. The sporophyll resembles that of Pinus, although it is comparatively shorter and broader, and has two abaxial sporangia (*fig. 1*). Whether it has any aborted sporangia, as reported in the Taxineae, could not be determined. At least when mature it has fewer sporangia than any of the Taxineae; *Taxus baccata* having seven, *Torreya taxifolia* four, Cephalotaxus four to two, and Phyllocladus two.

Male gametophyte

Material collected November 1 showed the microspores shed and the sporangia wide open along the whole line of dehiscence. Only three or four sporangia retained a few spores, and therefore the sections did not show many stages.

The youngest stage found in the development of the microspore was the uninucleate stage (*fig. 3*), with the wings fully developed. The microspores contain no starch; COULTER and LAND (9) found the microspores of *Torreya taxifolia* rich in starch; Miss YOUNG (20) found starch in the spores of Dacrydium; the body cell of *Cryptomeria japonica* contains starch grains, according to LAWSON (16); COKER (7) found the spores of Podocarpus, during all of the divisions, packed with starch, which disappears just before shedding; CHAMBERLAIN (6) found starch in the spores of *Pinus Laricio*, and Miss FERGUSON (12) in the spores of the species of pine studied by her.

The first division of the microspore cuts off a prothallial cell, which usually lies against the wall, and which immediately begins to disorganize (*fig. 6*). Soon a second prothallial cell is cut off. These two prothallial cells are at first surrounded by delicate walls which very soon disappear, so that when the spore is mature all the four nuclei lie free in the common cytoplasm. The first prothallial cell is commonly evanescent and its nucleus soon disintegrates (*figs. 9, 10*); in two mature spores it was still recognizable (*fig. 11*). The second prothallial cell always persists, in which respect Phyllocladus is similar to Ginkgo.

Prothallial cells have heretofore not been reported in any of the Coniferales except Podocarpineae and Abietineae. COKER (7) reports two prothallial cells in Podocarpus, one of which develops further, while the other slowly degenerates. In *Podocarpus Totarra*

Hallii BURLINGAME (4) finds two prothallial cells, both of which divide and form a tissue, consisting generally of six cells; a similar condition was found in Dacrydium by MISS YOUNG (**16**). Two more divisions follow, so that the mature spore commonly contains four free nuclei—prothallial, tube, stalk, and body nuclei.

On November 1 the four-nucleate microspores are mostly found lodged on top of the nucellus (*fig. 15*); how much earlier they reach it and how long they remain there is not known, as no material previous to this date was available. In exceptional instances pollen tubes had been formed and had penetrated the nucellus (*fig. 18*); but at this date the tube contained only the four nuclei of the mature spore. On December 11 the nucellus was in most cases entirely honeycombed with pollen tubes, and very much disintegrated (*fig. 19*). The pollen tubes do not pass deviously through the nucellus to any great extent, nor branch, but pass almost in a straight line to the embryo sac. As many as fifteen tubes were found in one ovule (*fig. 19*).

Before the pollen tube enters the embryo sac the body cell divides into two equal and optically similar male cells; it was impossible to observe this division, but it takes place some time after the tube has penetrated the nucellus and before it enters the embryo sac (*figs. 20, 31b*). The entire contents of the pollen tube (five naked nuclei) enter the egg (*fig. 20*). ARNOLDI (**1**) also finds that in Cephalotaxus the contents of the pollen tube are emptied into the embryo sac, the contents in this case, however, consisting of only four nuclei; a similar condition has been found in Pinus, where nearly the whole of the contents of the tube is emptied into the cytoplasm of the egg (COULTER and CHAMBERLAIN **10**, and FERGUSON **12**); according to COKER (**8**) this may also take place in Taxodium (**8**) and in Podocarpus (**7**); and COULTER and LAND (**9**) report it for *Torreya taxifolia.* In Cryptomeria LAWSON (**16**) finds that only one male cell enters the archegonium; and JÄGER (**13**) reports the same in *Taxus baccata.* In Phyllocladus it is not uncommon to find the entire contents of pollen tubes within the embryo sac after fertilization has taken place, and even after the eight-nucleate proembryo stage (*fig. 30*); this is no doubt due to the unusual open condition of the archegonia, the greatly disintegrated nucellus (which seems to be of a very mucilaginous consistency), and the large number of pollen tubes.

When the two male cells are equal, it is commonly inferred that both function. So far as could be determined, only one male cell functions in Phyllocladus, and the second male cell, together with the other three nuclei discharged into the egg cell from the pollen tube, disintegrate as the proembryo develops, as shown in *fig. 26*. ARNOLDI (**1**) finds in Cephalotaxus that the second male cell remains in the upper end of the egg and later goes through a mitotic division.

The Taxineae are equally divided in regard to the size of the male cells. In *Torreya taxifolia* they are unequal (COULTER and LAND **9**); they are also reported unequal in Taxus by BELAJEFF (**3**), and by JÄGER (**13**); ARNOLDI reports them of the same size in Cephalotaxus (**1**); and they are equal in Phyllocladus. Outside of the Taxineae they are equal in *Juniperus communis* (NORÉN **17**), Thuja (LAND **15**), Sequoia (ARNOLDI **2**), *Pinus Banksiana* (COULTER **11**), and *Pinus Laricio* (CHAMBERLAIN **6**). Miss FERGUSON (**12**) finds them unequal in *Pinus Strobus;* and one functioning male cell is reported in Podocarpus by COKER (**7**). LAND (**13**) finds in *Ephedra trifurca* that the male cells are equal, both enter the egg, but only one functions.

Ovulate strobilus

The ovulate strobilus occurs on the side of the phylloclad (in the axil of a scale leaf) near its base (*fig. 12*). It usually occurs singly, but frequently in pairs; never more than one pair was seen upon a single phylloclad. The strobilus consists of six to eight rather fleshy scales, each scale bearing a single ovule in its axis.

The ovule has two integuments, entirely free from the nucellus to the very base. The inner one is very thick and consists of three layers: an inner fleshy, a middle stony, and an outer fleshy consisting of only two layers of cells. The stony layer begins to develop at the micropylar end and extends toward the base. At first it consists of only one layer of cells, but later it becomes much thicker and extends all the way around the base of the ovule, where, however, it remains much thinner than at the micropylar end. The inner fleshy layer is somewhat crowded out by the growth of the stony layer; and the outer fleshy layer is finally sloughed off. The integument contains no vascular strands; these end at the base of the ovule (*fig. 15*); it contains a number of resin ducts, shown in the cross-section in *fig. 16*.

The second integument, called an arillus, arises quite late outside of the heavy integument; on November 1 it appears merely as a small papilla in longitudinal section (*fig. 15*). It grows very rapidly, inclosing the ovule like a cup; the ovule, however, grows up through it, and by December 11 it is merely a light, leathery sheath around the base of the ovule, reaching about half-way to its tip (*fig. 14*).

Female gametophyte

It was impossible from the material in hand to ascertain the development of the female gametophyte, as the formation of walls was in most cases well advanced on November 1. In a few instances it was still in the free nuclear stage (*fig. 18*); and one preparation showed archegonia already formed. The endosperm is of the "ruminating" type, this feature being especially pronounced in the early stages.

The development of archegonia could not be observed, as no stage before fertilization was available. It is impossible, therefore, to state definitely whether neck cells are formed or not. Some indefinite remains of cells, in a few sections, indicate that they exist; and comparing Phyllocladus with the Taxineae, and taking into consideration that neck cells are often destroyed very early by the pushing-in of the pollen tube or the growth of the central cell, the probability is that a two-celled neck exists in the early stages of the archegonium (*fig. 32b*), although nothing was found upon which a definite statement can be based. In *Torreya taxifolia* COULTER and LAND (**9**) found two neck cells; ARNOLDI (**1**) also found two in *Cephalotaxus Fortunei;* JÄGER (**13**) reports two in *Taxus baccata.* From such a condition as that shown in *figs. 19, 31, 32*, it may be inferred that the presence or absence of neck cells would make very little difference. The whole structure of nucellus and gametophyte is of a very mucilaginous composition, and this, together with the prodigality of pollen tubes and male cells, presents a very loose and disintegrated condition. The archegonium in every case, whether located near the surface of the gametophyte or deeper in the tissue, presents an opening as large as the width of the sac. The pollen tube penetrates the tissue, digesting it very readily, and seems to break through the layer of jacket cells surrounding the embryo sac as though no obstacle were in its way. No instance was found, at the stage of fertilization, where the archegonium had not been smashed into, either from the top or from the side, by the pollen tube.

The embryo sac is surrounded by a layer of jacket cells with very large nuclei, and many of the cells are multinucleate. JÄGER (13) also pictures a very heavy jacket layer of uninucleate cells in *Taxus baccata;* none are reported in Torreya or Cephalotaxus, although the drawings of the latter by ARNOLDI (1) suggest a jacket layer. The archegonia are one to four in number.

No ventral cell or nucleus could be demonstrated, although the probability is that a ventral nucleus is formed; the chromatin in the egg nucleus (*fig. 32b*) before fertilization indicates that it is getting ready to divide. ARNOLDI (1) says that in *Cephalotaxus Fortunei* the egg nucleus, shortly before fertilization, cuts off a ventral canal nucleus, which together with a mass of the upper part of the egg destroys the neck cells and passes out of the embryo sac. JÄGER (13) does not mention nor picture a ventral nucleus in Taxus; COULTER and LAND (9) did not find a ventral cell or nucleus in *Torrèya taxifolia;* Miss ROBERTSON (1) interpreted a spindle in the central cell of *Torreya californica* as the forming of a ventral nucleus.

Fertilization

At the time of fertilization the egg becomes rich in food vacuoles in the basal end (*figs. 25, 26, 28*). The egg nucleus may be situated near the upper end of the egg (*figs. 22, 23*), or near the basal end (*fig. 28*). The fusion nucleus (*figs. 22, 24*) is partially surrounded by the finely granular cytoplasmic sheath of the male nucleus. This cytoplasmic sheath has been observed in Taxodium by COKER (8), in *Torreya taxifolia* by COULTER and LAND (9), and in *T. californica* by Miss ROBERTSON (19). The non-functional male cell (*figs. 22, 23*), which has begun to disorganize, shows the cytoplasmic sheath very distinctly.

Embryo

The first division of the oospore could not be obtained. Free nuclear division was observed to the eight-nucleate stage. As this was the oldest stage available, it is impossible to say whether more free nuclei are formed or not before the formation of walls. ARNOLDI (1) figures ten free nuclei in one section of *Cephalotaxus Fortunei,* and states that there are eight to sixteen free nuclei. **Jäger** (13) found sixteen free nuclei in the proembryo of *Taxus baccata;* and **Coulter** and **Land** (9) found four free nuclei in *Torreya taxifolia.* One of the older ovules showed a long suspensor and an embryo of a few cells buried near the base of the gametophyte (*fig. 33*), but it was impossible to make out its structure.

Summary

The microsporophyll has two abaxial sporangia and the microspores are shed on November 1 or before.

Two prothallial cells are formed, the first of which is generally evanescent; sometimes both persist as free nuclei, the walls of both being evanescent.

The mature microspore has usually four free nuclei, and occasionally five.

The ovulate strobilus is borne on the phylloclad, and bears two to eight ovules.

The ovule has two integuments; one thick and fleshy, the other (arillus) thin and leathery and persistent only at the base. They are free from the nucellus to its base, and contain no vascular strands.

Microspores are found resting on the top of the nucellus on November 1, and occasionally pollen tubes and archegonia are formed at this date.

Pollen tubes are very numerous and pass in a comparatively straight line through the nucellus to the archegonium. They smash the neck cells, if there are any, and empty their entire contents into the egg; the contents of one or more pollen tubes were found in an egg after the eight-nucleate stage of the proembryo.

The body cell divides into two equal male cells just before discharge into the egg; and only one male cell functions.

The archegonium is surrounded by a heavy jacket layer, consisting of multinucleate cells with large nuclei. Two neck cells are probably formed. No ventral cell or nucleus was found, but the material does not justify a definite statement as to its occurrence or not.

The male cell is surrounded by a cytoplasmic sheath which, partly at least, surrounds the fusion nucleus.

At least eight free nuclei are formed by the proembryo before cell walls are formed; and a long suspensor is developed.

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EXPLANATION OF PLATES XX–XXII

With the exception of *figs. 1* and *2* all the figures were drawn with the aid of a camera lucida and reduced one-half. Index letters are as follows: *e*, endosperm; *p*, perisperm; *n*, nucellus; *if*, inner fleshy layer; *s*, stony layer; *of*, outer fleshy layer; *a*, arillus; *fs*, fleshy scale.

PLATE XX

FIG. 1.—Abaxial view of microsporophyll, showing the empty sporangia.

FIG. 2.—Side view of microsporophyll.

FIG. 3.—Microspore. ×1850.

FIG. 4.—First division of microspore. ×1850.

FIG. 5.—Two-nucleate stage. ×1850.

FIG. 6.—Second division; first prothallial cell against upper wall. ×1850.

FIG. 7.—Three-nucleate stage, showing form of wings. ×3000.

FIG. 8.—Evanescent walls of the two prothallial cells. ×3000.

FIG. 9.—First evanescent prothallial cell lying outside of the cytoplasm of the spore. ×1850.

FIG. 10.—Mature microspore. ×1850.

FIG. 11.—Microspore with both prothallial cells retained. ×3000.

FIG. 12.—Phylloclad with ovulate strobilus.

FIG. 13.—Outer view of fleshy scale with ovule.

FIG. 14.—Inner view of fleshy scale with ovule, showing arillus.

FIG. 15.—Longitudinal section of ovulate strobilus, showing ovules with integuments, fleshy scales, and position of vascular system, November 1. ×40.

FIG. 16.—Cross-section of ovule; archegonia in the center of the endosperm; November 1. ×40.

FIG. 17.—Longitudinal section of the ovule with fleshy scales; micropyle not yet formed; arillus covering the ovule; November 1. ×40.

PLATE XXI

FIG. 18.—Part of nucellus on November 1, showing pollen tubes and part of the female gametophyte in the free nuclear stage; archegonia not yet formed; body cell not yet divided. ×850.

FIG. 19.—Nucellus showing numerous pollen tubes and open archegonia; nearly all the body cells have divided; December 11. ×180.

FIG. 20.—Pollen tube, containing two male nuclei, the stalk and tube nuclei, and one prothallial nucleus, entering the archegonium. ×1850.

FIG. 21.—Archegonial jacket cells. ×3000; *fig. 21a*, same.

FIG. 22. Fertilization: the fusing nuclei are partially surrounded by the cytoplasmic sheath; the second male nucleus lies above the fusing nuclei and has begun to degenerate. ×3000.

FIG. 23.—A later stage in fertilization. ×3000.

FIG. 24.—Fusing nuclei with cytoplasmic sheath. ×1850.

FIG. 25.—Second division of proembryo. ×3000.

FIG. 26.—Four-nucleate stage of proembryo; the other four nuclei degenerating. ×3000.

FIG. 27.—Section through thick integument showing inner fleshy layer, the row of heavy-walled cells (containing crystals) which becomes the stony layer, and the outer fleshy layer of two rows of cells. ×1850.

PLATE XXII

FIG. 28.—Basal end of egg, showing egg nucleus, pollen tube with two male nuclei and one other nucleus, and food vacuoles. ×1850.

FIG. 28*b*.—The archegonium of *fig. 28*, showing position of egg nucleus and pollen tube in the egg. ×100.

FIG. 29.—Basal end of embryo sac, showing four nuclei of the proembryo, remains of the pollen tube, and the non-functional male cell. ×1850.

FIG. 29*b*.—Same as *fig. 29*, showing whole length of embryo sac. ×100.

FIG. 30.—Eight free nuclei of proembryo in basal end of sac, with pollen tube above containing normal contents. ×1850.

FIG. 30*b*.—Same as *fig. 30*, showing top of embryo sac. ×100.

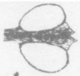
FIG. 31.—Female gametophyte, showing disintegration by pollen tube; the pollen tube is entering the egg from the side. ×100.

FIG. 31*b*.—Same as *fig. 31*. ×1850.

FIG. 32.—This figure shows the straight path usually made by the pollen tubes through the nucellus to the archegonia. ×100.

FIG. 32*b*.—Same as *fig. 32*, showing the two probable neck cells; the loose structure of the chromatin of the egg nucleus intimates that it may form a ventral nucleus; body cell not yet divided. ×1850.

FIG. 33.—Long suspensor, with embryo buried deep in the female gametophyte. ×100.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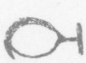
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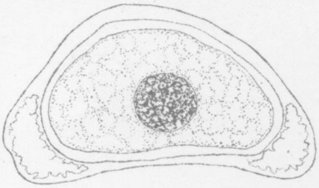
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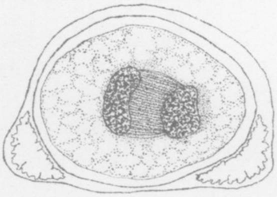
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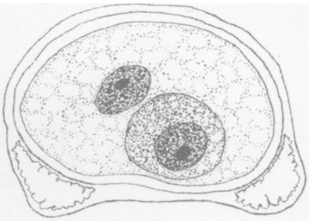
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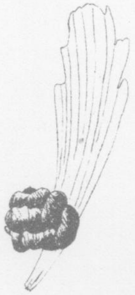
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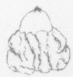
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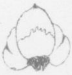
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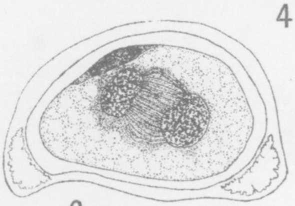
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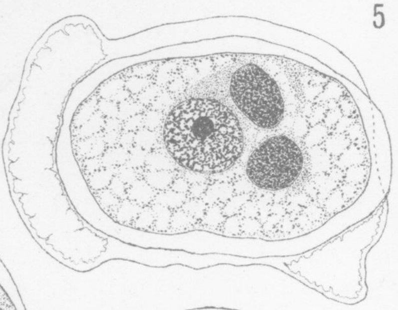
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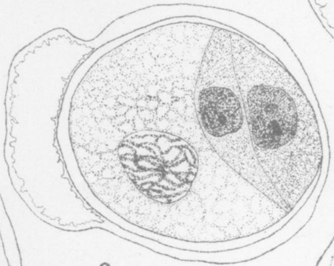
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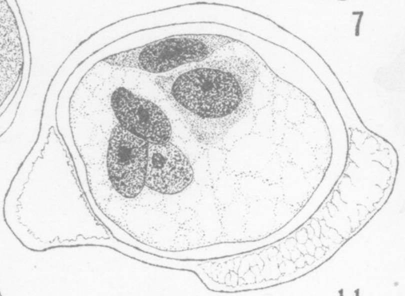
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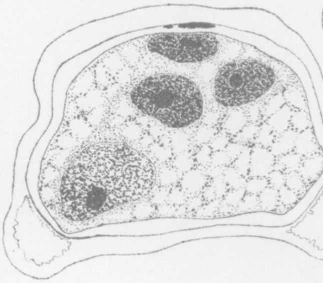
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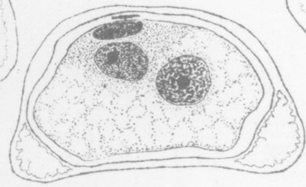
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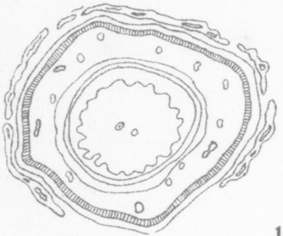
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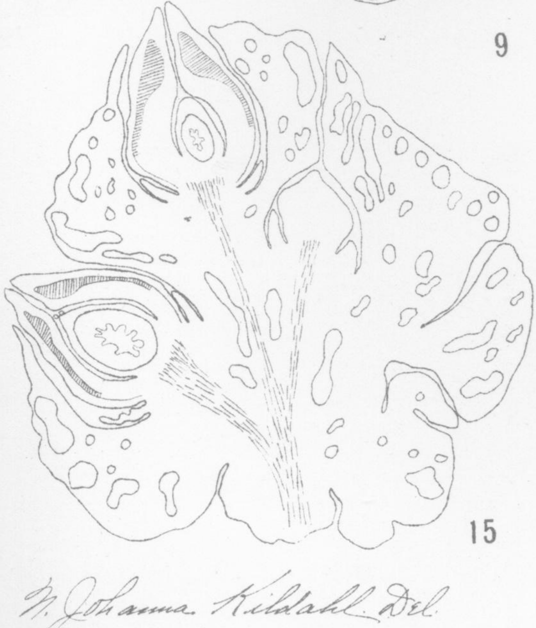
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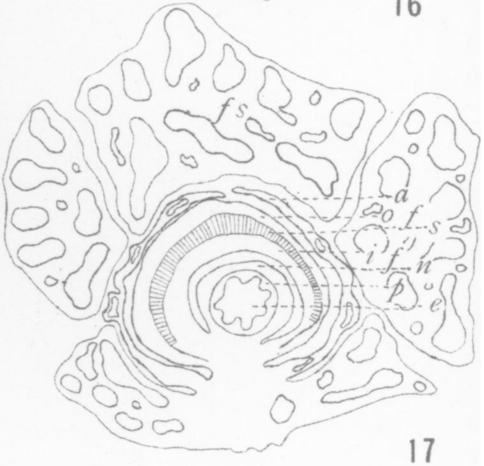

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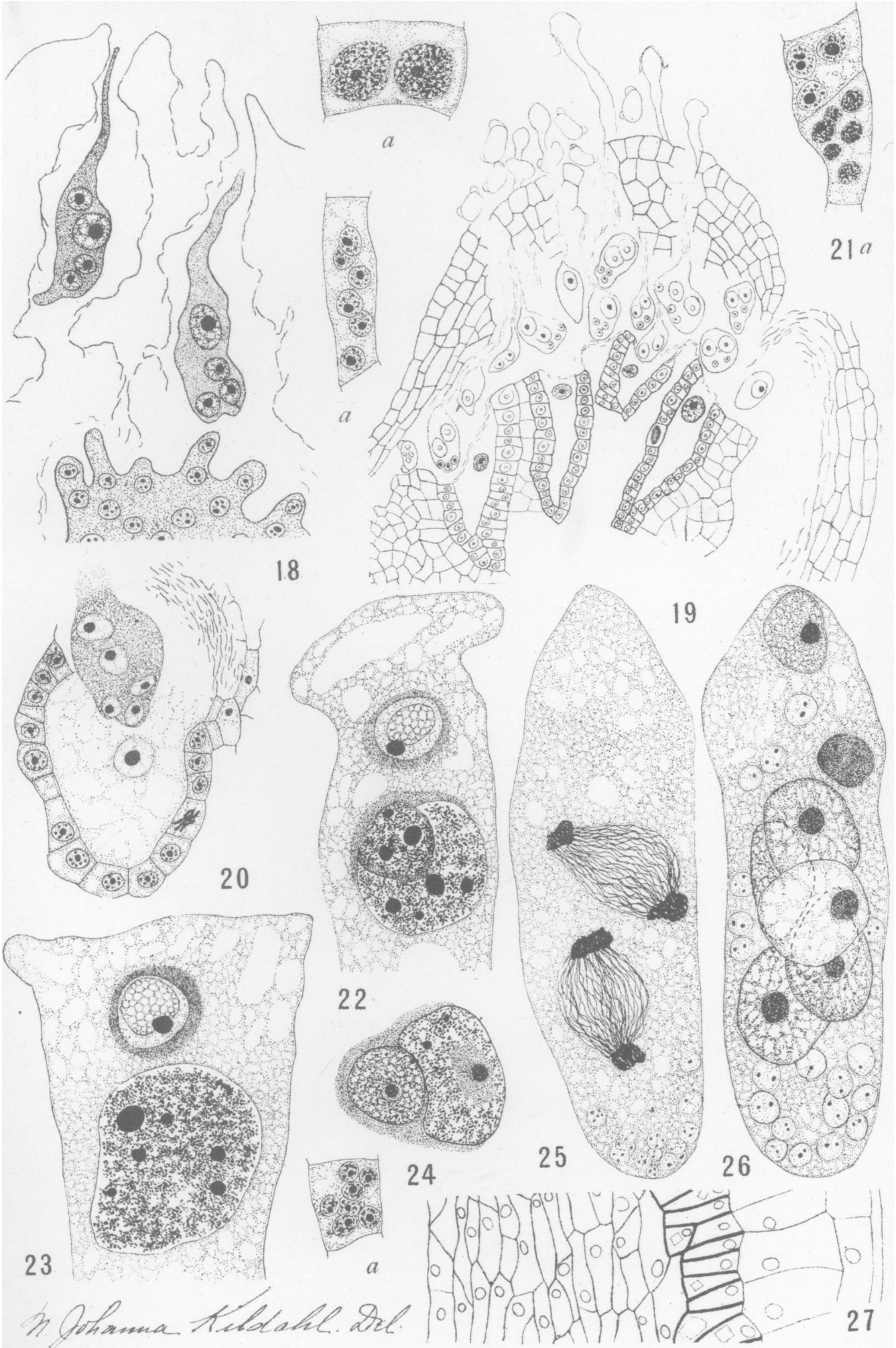
N. Johanna Kildahl Del.

KILDAHL on PHYLLOCLADUS



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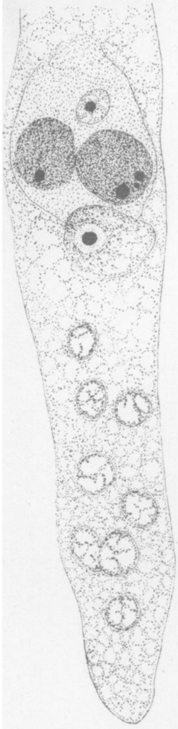

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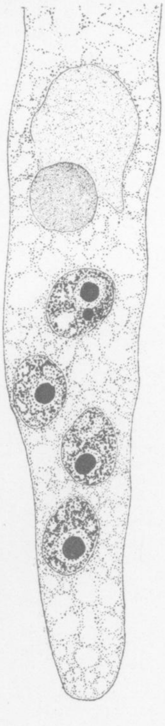

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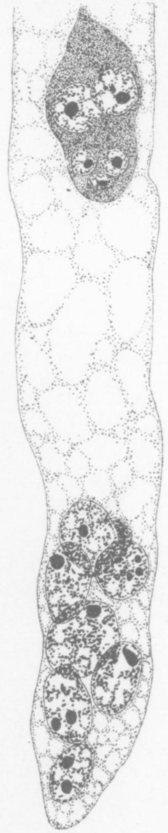

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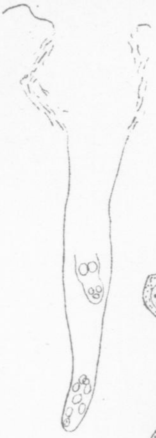

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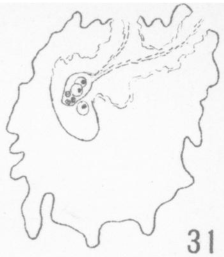

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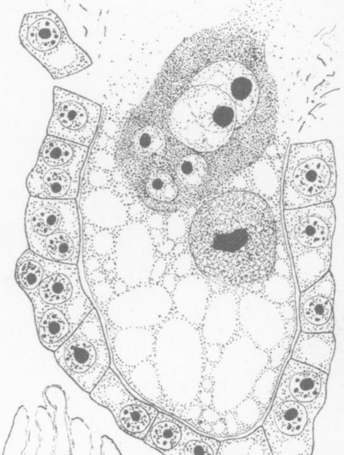

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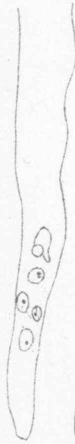

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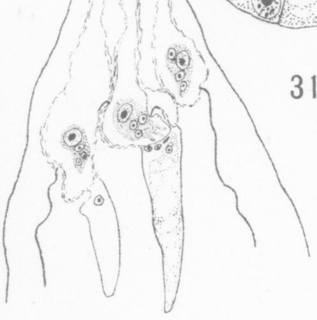

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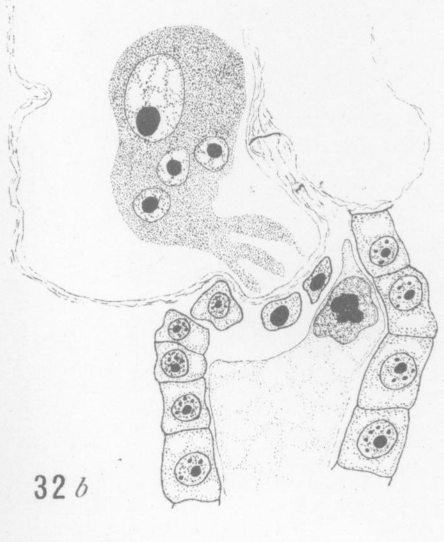

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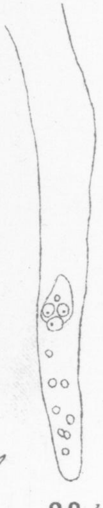

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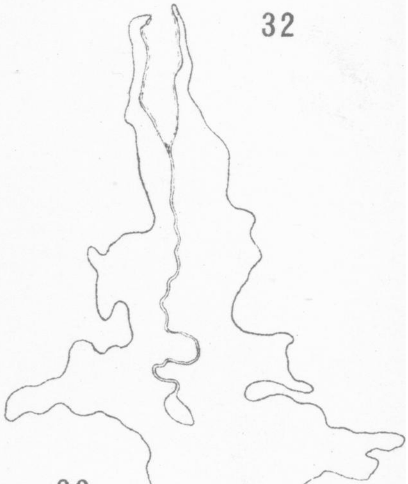

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